Optimisation of biogas production from the macroalgae Laminaria sp. at different periods of harvesting in Ireland

Montingelli, Maria; Benyounis, Khaled; Quilty, Brid ; Stokes, Joseph; Olabi, Abdul-Ghani

Published in:
Applied Energy

DOI:
10.1016/j.apenergy.2016.05.150

Published: 01/09/2016

Document Version
Peer reviewed version

Optimisation of biogas production from the macroalgae *Laminaria sp*. at different periods of harvesting in Ireland

Maria E. Montingelli[1,a], Khaled Y. Benyounis[1], Brid Quilty[2], Joseph Stokes[1], Abdul G. Olabi[3]

[1]Department of Mechanical and Manufacturing Engineering, Dublin City University, Dublin, Ireland,
[a] Corresponding author, e-mail: maria.montingelli3@mail.dcu.ie
[2]Department of Biotechnology, Dublin City University, Dublin, Ireland
[3]School of Engineering, University of the West of Scotland, Paisley, Scotland, UK

ABSTRACT

Brown seaweeds are a suitable substrate for biogas production through anaerobic digestion (AD). Since the carbohydrates' content is subjected to a seasonal variation, this research aimed to select the best harvesting period of beach cast brown seaweed *Laminaria sp*. for methane production, while optimising the use of a beating pretreatment and the organic substrate concentration. Response surface methodology (RSM) was used to estimate the effect of the beating pretreatment in terms of pretreatment time (5-10-15 min) and organic matter concentration in terms of volatile solids (VS) (1-2.5-4%) on methane production. The highest methane yield of 342 ± 17 ml g^{-1}VS was observed during November at 1% of VS and after 5 min of pretreatment, while the lowest yields were registered in March with an average of 163 ± 28 ml g^{-1}VS. However, an enhancement of 47% with respect to the untreated sample was achieved at 2.5% of VS and after 15 min of pretreatment, in March.

KEYWORDS

Laminaria sp., anaerobic digestion, biogas, pretreatment, organic substrate concentration, response surface methodology.

1. INTRODUCTION

First generation biofuels are made by using food crops as main feedstocks. The greenhouse gas savings associated with first generation biofuel systems could be negated by indirect land-use change (ILUC) emissions. These emissions occur when grassland and forest are converted to crop land somewhere on the globe to meet the demand for commodities displaced by the production of biofuel feedstocks [1]. The growth of terrestrial crops for biomass requires the use of significant amounts of land and water and can have implications for biodiversity, food production and landscape [2].

Second generation biofuels produced by lignocellulosic biomass, agricultural, municipal and industrial waste can mitigate the issues related to the first generation of biofuels. However, it has been highlighted [3] that the production of second-generation biofuel requires most sophisticated processing production equipment, thus requiring more investment per unit of production and larger-scale facilities to confine capital cost scale economies.

Third generation biofuels derived from algal biomass (micro- and macroalgae) are a valuable alternative to overcome the obstacles related to the first and second generation biofuels. This

kind of biomass ensures high growth yields without requiring arable land [4-6], high capacity of carbon capture during photosynthesis [7] and a negligible amount of lignin avoids the need for energy-intensive pretreatments [8].
In particular, macroalgae, commonly known as seaweeds, can be converted into biogas (~ 60% methane) via anaerobic digestion (AD) [9]. Biogas can then either be used to produce heat and electricity in combined heat and power (CHP) systems or upgraded to biomethane. Biomethane is a gas chemically identical to natural gas which can be injected into the gas grid or used as a transport fuel [10]. Compared to natural gas, algal biogas through AD has the potential to decrease greenhouse gas (GHG) emissions over 50% and fossil depletion of almost 70% [11].
In Ireland, several studies [12-14] assessed the use of grass as resource for biomethane production through AD. These studies are relevant since grass is the main Irish biomass used for ruminant production. Even though, grass was proven to contribute significantly to biogas production [15], the main concern is its competition with the Irish agricultural system [16]. Unlike grass, seaweed biomass do not compete with any Irish agricultural system and it offers higher gross energy yields of biomethane (365 GJ ha^{-1} yr^{-1}) with respect to grass biomethane (122-163 GJ ha^{-1} yr^{-1}) [17]. It represents an indigenous resource which can help the development of rural costal economy [7, 18] as well as an opportunity for the Irish marine sector [19]. Ireland has a long maritime tradition and significant potential for exploitation of marine resource. Most of the Irish seaweeds production is for the hydrocolloid industry and a significant quantity is sold as raw material for further industrial processing [20]. The estimated standing kelp stock is three million tonnes; although this estimation is highly uncertain [20]. However some obstacles need to be overcome. Although AD from seaweeds is technically proven, the optimisation of the process is still under research [21]. Efficient cultivation and harvesting are prerogatives in order to exploit the full potential of macroalgae especially on a larger scale for biofuel production [2, 7, 18, 22]. Several studies analysed the economics and the main advantages and drawbacks related to the use of seaweeds for biofuels along the entire supply chain [23, 24].
The use of seaweeds for biogas production through AD has been evaluated by several works in the literature [21, 25], with yields of up to 400 ml g^{-1}VS of methane in the case of brown seaweed *Laminaria sp*. [26]. The methane yields can be optimised by using a pretreatment step prior to AD. In particular, the use of a mechanical pretreatment can be a viable route for seaweed [21]. The main effect of a mechanical pretreatment is to facilitate the hydrolysis phase by increasing the substrate specific surface area and thus an increased access for degrading enzymes [27]. In general, the main drawback related to this kind of pretreatment is the high energy demand that in the case of seaweed it is believed to be lower due to the lack of lignin [28]. The beating pretreatment for seaweed biomass was investigated by Tedesco *et al.* [29] who showed an enhancement in biogas and methane yields of 52% and 53% respectively from *Laminaria sp*. While, considering different mechanical pretreatments, Montingelli *et al.* [30] showed that beating pretreatment on *Laminaria sp*. was able to promote the start of the AD and reduce the incubation time while microwave and ball milling pretreatment had a negative effect on methane yields.
Amongst all the parameters which influence the biogas yields, the substrate concentration is one of the most crucial [31]. It is known that an excessive substrate concentration leads to imbalances in the bacterial population, leading to VS accumulation and digester failure [32]. On the other hand, excessively low substrate concentration can result in starving conditions within the digester and a consequent reduced methane generation [33]. In the case of seaweeds, suitable substrate concentration must be investigated according to the nature and composition of the seaweed species [21].

In the case of seaweed biomass, gas yields are also related to the level of storage sugars; and, as these vary with season, gas yields will vary [7]. It is necessary that seaweed is harvested when the sugar content is highest, making the seasonality an important factor affecting the economics of the system [34]. However, very few studies in the literature investigated the methane production through AD from *Laminaria sp*. at different periods of harvesting, in Ireland [35].

Considering the beneficial effect of beating pretreatment on *Laminaria sp*. [29, 30], this experimental study evaluated the impact of beating pretreatment and substrate concentration on AD of the brown seaweed *Laminaria sp*., at different harvesting times, in Ireland. The pretreatment phase was tested in terms of beating time, while the substrate concentration was considered in terms of VS concentration. The response surface methodology (RSM) approach was used. This method allowed evaluating the possible interaction of influencing parameters on AD by limiting the number of planned experiments. The aim was to identify the best period of harvesting in Ireland, in conjunction with the best condition of organic substrate concentration and pretreatment phase. An energy evaluation for each harvesting period was also carried out.

2. MATERIALS AND METHODS

2.1 Feedstock and inoculum

Samples of a mixture of *Laminaria sp*. (*Laminaria digitata*, *Saccharina latissima* and *Laminaria hyperborea*) were manually collected on shore in Howth (Dublin, Ireland), in order to reproduce the case of harvesting readily biomass available on the beach. The biomass was harvested three times along a year, in the following format: in early May 2014, a sample was collected as representative of the end of spring and start of summer season; in November 2014, a sample was collected as representative of the end of autumn and start of winter season; in March 2015, a sample was collected as representative of the end of winter and start of spring season.

For each month, before beating pretreatment, fresh seaweeds were roughly cut without washing. For each collection, Table 1 reports the total solids (TS) and volatile solids (VS) analysis of the mixture of *Laminaria sp*.

Table 1: TS and VS analysis

Period	*TS (% Wt on wet basis)*	*VS (% Wt of TS)*
May 2014	14±1	66±8
November 2014	19±2	84±1
March 2015	13±2	74±1

Sewage sludge was used as source of inoculum [36]. The sludge was collected from the Ringsend wastewater treatment plant (Celtic Anglian Water Ltd.), Dublin, Ireland operating at mesophilic temperature. Once collected from the plant, the inoculum was immediately used and not allowed to degasify. Sludge-only samples were digested and the amount of biogas produced was subtracted from the seaweed-sludge yields. The analysis revealed total solids (TS) content of 3.6 ± 0.5% Wt on its wet basis, a volatile solids (VS) concentration of 79.5 ± 4% Wt on its dry basis and a pH value equal to 8.0 ± 0.1. The Total COD (Chemical Oxygen Demand) and Soluble COD were found equal to 60.15 ± 6.8 g O_2 L^{-1} and 5.8 ± 0.42 g O_2 L^{-1} respectively

2.3 Anaerobic biodegradability

A batch system, as reported in [30], was used as the AD experiment set-up. The bioreactors consisted of borosilicate glass flasks of 500 ml in capacity. Each bioreactor was filled with 200 ml of treated seaweed and tap water with different amounts of seaweed, in order to reach the VS concentrations under investigation (1%, 2.5% and 4%,) and 200 ml solution of inoculum and tap water at a constant VS concentration of 3%. In particular, the loading of the beating machine was adjusted in order to have an algal VS concentration of 4% of the total machine's working weight. Samples were then adjusted by water dilution in order to achieve the desired concentration. Each bioreactor had a total working volume of 400 ml. Thus, the Food to Inoculum ratios (F:I) tested were 0.34, 0.83 and 1.34 for the bioreactor at 1%, 2.5% and 4% respectively.

After inoculum addition, the pH for each sample was measured by using a Hanna precision pH meter (accuracy ± 0.01), model pH 213. All the samples exhibited an initial pH in the range between 7 and 8 ± 0.01. Samples of untreated seaweed for each different VS concentration tested were also included. Samples of sludge-only were prepared with 200 ml of inoculum and 200 ml of tap water in order to obtain a total working volume of 400 ml. The amount of biogas produced by sludge-only samples was then subtracted from the co-digesting yields in order to evaluate the algal contribution. All samples were carried out in duplicates. The reactors were then sealed with borosilicate glass adapters equipped with controlled gas opening valves and purged with nitrogen flow for 5 minutes in order to achieve anaerobic conditions. The incubation time was set at 14 days. The biogas produced during the digestion was collected in airtight Linde plastic-gas bags and measured after 6 days and at the end of digestion. At each collection the biogas volume was measured by using gas sampling tubes which were installed in a gas jar with confining liquid according to procedure VDI 4630 [36]. Water baths were used to incubate the reactors at an operating mesophilic temperature of 38 ± 1°C. During incubation, the bioreactors were shaken manually once a day. A biogas analyser, model Drager X-am 7000, was used to verify that the system was anaerobically isolated, and to measure the percentages of CH_4 and CO_2 in the biogas.

2.4 Response surface methodology (RSM)

A face-centred central composite design (FCCD) involving two numeric factors (A: time of pretreatment and B: VS concentration) was adopted as RSM. This design was replicated three times over a year, according to different harvesting months as reported in *Section 2.1*. The levels' values for each variable were set as shown in Table 2.

Table 2: Variables matrix

Variable under investigation-Factor	*Levels*		*Response*
A: VS concentration [%]	1.	1%	Methane production
	2.	2.5%	[ml g^{-1} VS]
	3.	4%	
B: Beating time [min]	1.	5 min	
	2.	10 min	
	3.	15 min	

The levels' values were selected by considering previous studies on the subject [29, 31]. According to these studies, a centre point at 10 min and 2.5 % of VS concentration was also selected. Therefore, a total of 13 experiments were carried out for each harvesting month, with the first 9 experiments organized in a 3^2 full factorial design and the remaining 4 involving the replications of the centre point. The analysis of variance (ANOVA) was used in order to check the adequacy of the model developed and to obtain the interaction between the process variables and the response. The quality of the polynomial model fit was expressed by the coefficient of determination R^2, and its statistical significance was checked by the Fisher's *F*-test. Model terms were evaluated by the *p*-value with 95% confidence level ($\alpha = 0.05$). The statistical analysis was carried out by using the Design-Expert software (version 9.0.3.1).

2.5 TS and VS analysis

The TS amount was determined by drying the samples at 105°C until constant weight, while the VS fraction was assessed through combustion of a known weight dried sample at 575 ± 25°C overnight, according to standard methods (NREL/MRI LAP 1994, 2008) [37, 38].

2.6 Beating pretreatment

Beating was performed as mechanical pretreatment using a Hollander beater, model Reina. This kind of machine was originally built for the pulp and paper industry. It was equipped with a crank handle which allowed adjustment of the gap between the drum’s blades and the bed-plate. The minimum gap achievable was 76 µm, which corresponded to one single turn of the crank handle. In general, the machine performs two main actions; (a) - cutting action caused by the grooves located on the bed-plate, and (b) - high pressure beating action of the feedstock against an inclined plate placed at the exit-out of the drum. The drum of the machine permitted a constant rotational speed of 580 rpm. Even though, the machine was capable to operate both wet and dry biomass, it was necessary to add tap water in order to cause the recirculation of the feedstock. The result was a pulp of different consistencies according to the gap and the processing time applied. In this experimental work, the machine was operated at the minimum gap of 76 µm for each level of beating time (5, 10, 15 min) under investigation.

2.7 Total and soluble COD

Total (tCOD) and soluble COD (sCOD) were determined through the colorimetric method. For COD analysis the procedure followed is reported as Method 8000 for water, wastewater and seawater by Hach Lange Company. The measurements were carried out using Hach standard kit (range 0-1500 mg L^{-1}, Hach Lange, Düsseldorf, Germany) and a Hach Lange DR2000 spectrometer to read the samples. Prior to sCOD determination, a vacuum filtration through a glass microfiber filter (1.5 µm of pore size) at first and then through a membrane filter (0.1 µm of pore size) was performed. Both tCOD and sCOD were determined by diluting the samples at a dilution factor of 1:100. The initial and final sCOD were measured before the samples were subjected to anaerobic conditions and at the end of the incubation time respectively.

2.8 Energy balance calculation method

The following formulas, as reported in [30], were employed in order to calculate the energy balance related to the use of the mechanical pretreatment.

$$B_S = CH_4\,[\%] * (9.67/97) \qquad (1)$$

In the above equation, B_s [kWh m^{-3}] is the energy content of the biogas produced by seaweed and CH_4 [%] is the average methane content of the biogas produced by seaweed at each collection. A reference value of 9.67 kWh at 97% of methane was used [39, 40], in order to calculate the energy content of the biogas produced.

$$E_p = B_p * B_s \tag{2}$$

In the above equation, E_p [Wh g^{-1}VS] is the energy generated from the biogas produced from 1 g of VS of seaweed and B_p [m^3 g^{-1}VS] is the quantity of biogas produced for each gram of VS of seaweed.

$$E_C = E_{pt}/VS_m \tag{3}$$

In the above equation, E_C [Wh g^{-1}VS] is the electricity consumed by the pretreatment in order to process 1 g of VS of seaweed, E_{pt} [Wh] is the electricity consumed during the pretreatment measured by a kilowatt hour meter (Prodigit Model 2000MU-UK plug-in power, accuracy 0.5%), VS_m [g] is the total amount of VS into the machine.

$$Net\ E_P = E_P - E_C \tag{4}$$

In the above equation, the $Net\ E_P$ [Wh g^{-1}VS] is the energy produced by 1 g of VS of seaweed treated. In the case of untreated seaweed the E_C term was equal to zero since no mechanical pretreatment was applied.

$$Energy\ Gain\ [\%] = \frac{(Net\ E_P)_{pretreatment} - E_{P_{untreated}}}{E_{P_{untreated}}} * 100 \tag{5}$$

The *Energy Gain* [%] is the difference in percentage between the energy provided by the biogas produced from treated seaweed $(Net\ E_P)_{pretreatment}$ and the energy from the biogas provided by the untreated seaweed $E_{Puntreated}$. The *Energy Gain* was negative when the $E_{Puntreated}$ term is > than the $(Net\ E_P)_{pretreatment}$ term, which meant that the use of the pretreatment caused a loss of energy compared to the case of untreated seaweed.

3. RESULTS AND DISCUSSION

3.1 Methane production

In general, the results showed that the effect of pretreatment is different according to the harvesting period and the VS concentration. Tables 3-4-5 and **Figure** 1 report the cumulative methane yields achieved when the seaweed was subjected to AD for 14 days at different experimental combinations in May 2014, November 2014, and March 2015. The best results were achieved when the material was harvested in November 2014 (Table 4, Figure 1-b). In this period, the highest methane yield recorded was 342 ± 17 ml g^{-1} VS after 5 min of pretreatment and at a VS concentration of 1%. This corresponded to 59% and 43% more methane compared to the best yields achieved in March and May respectively. In the same period, an average of 220 ± 26 ml CH_4 g^{-1} VS was registered for the other experimental conditions. At 2.5% of VS a general enhancement between 19-28% was observed, while negligible enhancements were recorded at 4% of VS with respect to the untreated samples.

The lowest methane yields were registered in March (Table 5, Figure 1-c). In particular, at 4% of VS a failure of the digester was observed since negligible levels of methane and very high percentages (70-80%) of CO_2 were detected. This was also confirmed by the high levels of sCOD registered at 14 days of digestion as well as an average pH of 6.71 ± 0.04, which was too low in order to allow methane production. These were all signs of an unbalanced digestion caused by an overloading of the digester [41]. On the other hand, the other samples exhibited an average of 163 ± 28 ml CH_4 g^{-1} VS, with a peak of 215 ± 9 ml CH_4 g^{-1} VS. Even though March was characterised by the lowest yields, a general enhancement of methane after beating pretreatment was recorded. Samples at 1% of VS showed an increase of methane with respect to the untreated sample from 13% up to 22%, while the best methane enhancement of 47% was achieved at 2.5% of VS and after 15 min of pretreatment. Results from May (Table 3, Figure 1-a) suggested that the use of a pretreatment step did not allow a high enhancement of methane yield during this period. The seaweed harvested during this month showed around 9% more methane than the untreated samples, only at 2.5 % of VS and after 10 and 15 min of beating.

Table 3: Methane, Biogas yields, sCOD in May 2014

Sample *VS [%]*	*BT [min]*	*Initial sCOD [g O_2 L^{-1}]*	*Final sCOD [g O_2 L^{-1}]*	*CH_4 [ml g^{-1} VS]*	*Biogas [ml g^{-1} VS]*
1	0	N.A.	N.A.	236±6	482±8
1	5	5.08±0.48	2.7±0.33	167 ± 23	402±20
1	10	4.78±0.28	2.08±0.38	210 ± 7	491±10
1	15	5.03±0.36	2.68±0.27	201 ± 20	463±25
2.5	0	N.A.	N.A.	221±26	451±24
2.5	5	5.63±0.61	2.80±0.46	208 ± 5	433±1
2.5	10	6.30±0.21	2.93±0.29	238 ± 20	494±22
2.5	15	5.53±0.96	2.2±0.55	240 ± 8	615±7
4	0	N.A.	N.A.	217±20	413±18
4	5	7.60±0.39	5.8±0.26	86 ± 12	222±23
4	10	7.53±1.13	4.63±0.49	139 ± 22	317±26
4	15	7.08±0.79	4.58±0.68	185 ±17	374±25

Table 4: Methane, Biogas yields, sCOD in November 2014

Sample *VS [%]*	*BT [min]*	*Initial sCOD [g O_2 L^{-1}]*	*Final sCOD [g O_2 L^{-1}]*	*CH_4 [ml g^{-1} VS]*	*Biogas [ml g^{-1} VS]*
1	0	N.A.	N.A	138±15	345±11
1	5	4.70±0.21	3.32±0.20	342±17	855±25
1	10	4.31±1.32	3.39±0.04	283±26	708±15
1	15	3.41±0.21	3.08±0.01	197±14	493±20
2.5	0	N.A	N.A.	172±20	430±22
2.5	5	8.23±0.53	3.46±0.03	220±3	523±6
2.5	10	10.15±0.39	2.91±0.02	207±7	467±21
2.5	15	9.41±0.56	3.26±0.38	204±10	493±8

4	0	N.A.	N.A.	209±17	502±20
4	5	11.75±0.84	3.67±0.06	212±17	512±12
4	10	12.43±0.28	3.55±0.23	202±23	485±21
4	15	12.30±0.39	3.10±0.15	212±16	514±5

Table 5: Methane, Biogas yields, sCOD in March 2015

Sample *VS* *[%]*	*BT* *[min]*	*Initial sCOD* *[g O_2 L^{-1}]*	*Final sCOD* *[g O_2 L^{-1}]*	*CH_4* *[ml g^{-1} VS]*	*Biogas* *[ml g^{-1} VS]*
1	0	N.A.	N.A	139±10	490±22
1	5	6.48±0.15	4.75±0.35	157±13	506±16
1	10	6.04±0.02	3.5±0.70	182±11	564±23
1	15	6.20±0.03	2.35±0.25	169±7	533±19
2.5	0	N.A.	N.A.	146±3	418±23
2.5	5	9.43±0.01	3.45±0.85	120±6	314±12
2.5	10	9.88±0.02	4.20±0.50	177±15	540±7
2.5	15	8.80±0.08	1.85±0.05	215±9	576±20
4	0	N.A.	N.A.	20±5	269±24
4	5	12.78±0.20	16.15±1.05	20±5	228±19
4	10	12.35±0.13	18.45±0.25	25±3	224±6
4	15	12.80±0.38	14.25±0.65	15±3	227±23

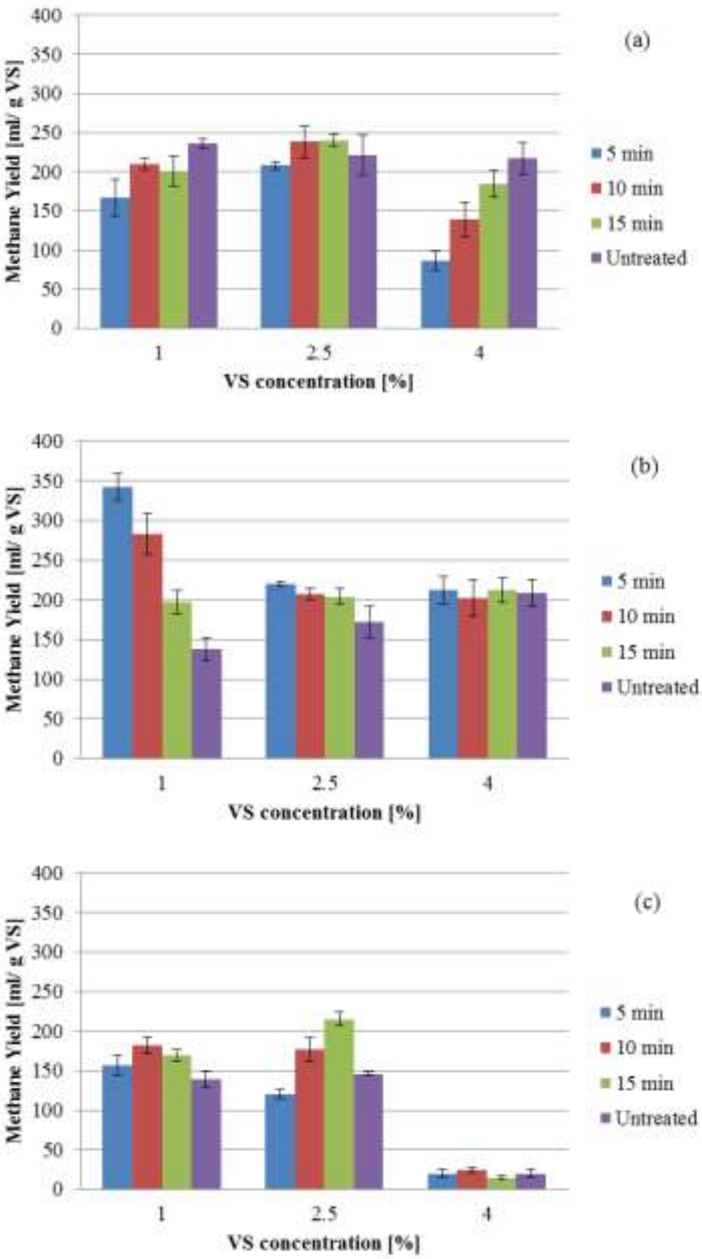


Figure 1: Methane yields in May 2014(a); 3, November 2014(b); 4, March 2015(c)

3.2 Model estimation

The ANOVA (Tables 6, 7, 8) for each period estimated that the models adopted were significant. According to the harvesting period, different terms were estimated significant. It is worth noting that for all periods the A (VS concentration) and A^2 terms were estimated significant, while the B term (beating time) was significant only in May and a significant interaction AB was found only in November. For each experiment, the estimated model was able to fit the data since the 'Lack of Fit' *p*-value was <0.05. Also the values of R^2, adjusted R^2 (*Adj.* R^2) and predicted R^2 (*Pred.* R^2) were all close to 1, indicating good regression models.

Table 6: ANOVA May 2014

Source	*Sum of Squares*	*df*	*Mean Square*	*F Value*	*p-value Prob > F*	
Model	28262.97	5	5652.59	20.76	0.0005	significant
A: VS [%]	4704.00	1	4704.00	17.28	0.0043	
B: BT [min]	4537.50	1	4537.50	16.66	0.0047	
AB	1056.25	1	1056.25	3.88	0.0895	
A^2	12194.84	1	12194.84	44.79	0.0003	
B^2	793.34	1	793.34	2.91	0.1316	
Residual	1905.96	7	272.28			
Lack of Fit	577.96	3	192.65	0.58	0.6585	Not significant
Pure Error	1328.00	4	332.00			
Cor Total	30168.92	12				

R^2 =0.9368; Adj. R^2 =0.8917; Pred. R^2=0.7421; Adeq. Precision=14.781

Table 7: ANOVA November 2014

Source	*Sum of Squares*	*df*	*Mean Square*	*F Value*	*p-value Prob > F*	
Model	20541.20	5	4108.24	63.08	<0.0001	significant
A: VS [%]	6402.67	1	6402.67	98.31	<0.0001	
B: BT [min]	128.00	1	128.00	1.97	0.2037	
AB	5256.25	1	5256.25	80.71	<0.0001	
A^2	3498.03	1	3498.03	53.71	0.0002	
A^2B	1064.08	1	1064.08	16.34	0.0049	
Residual	455.88	7	65.13			
Lack of Fit	223.88	3	74.63	1.29	0.3931	Not significant
Pure Error	232.00	4	58.00			
Cor Total	20997.08	12				

R^2 =0.9783; Adj. R^2 =0.9628; Pred. R^2=0.8538; Adeq. Precision=26.643

Table 8: ANOVA March 2014

Source	*Sum of Squares*	*df*	*Mean Square*	*F Value*	*p-value Prob > F*	
Model	55938.59	4	13984.65	30.73	<0.0001	significant
A: VS [%]	33450.67	1	33450.67	73.50	<0.0001	
B: BT [min]	1734.00	1	1734.00	3.81	0.0867	
A^2	15621.55	1	15621.55	34.33	0.0004	
B^2	346.88	1	346.88	0.76	0.4081	
Residual	3640.64	8	455.08			
Lack of Fit	2917.84	4	729.46	4.04	0.1026	Not significant
Pure Error	722.80	4	180.70			
Cor Total	59579.23	12				

R^2 =0.9389; Adj. R^2 =0.9083; Pred. R^2=0.7768; Adeq. Precision=13.898

For each group of data the software yielded the following model equations in terms of coded factors (Table 9).

Table 9: Variables' coded factors

	Coded factors		
Variable	-1	0	+1
A: VS concentration [%]	1	2.5	4
B: Beating time [min]	5	10	15

Each equation showed the methane yield (Y) as a function of the independent variables A (VS concentration) and B (beating time) for the experiment in May (Eq. 6), November (Eq. 7) and March (Eq. 8) respectively.

$$Y = + 242.41 - 28.00 A + 27.50 B + 16.25 AB - 66.45 A^2 - 16.95 B^2 \quad (6)$$

$$Y = + 208.43 - 32.67 A - 8.00 B + 36.25 AB + 32.90 A^2 - 28.25 A^2B \quad (7)$$

$$Y = + 177.34 - 74.67 A + 17.00 B - 75.21 A^2 - 11.21 B^2 \quad (8)$$

By considering the coefficients of each equation, it is possible to notice that the extent of impact for each term was different according to the harvesting time. In May (Eq. 6) the highest impact was represented by the quadratic term A^2, while the impacts on methane yield of the A (VS concentration) and B (beating time) terms had the same magnitude. In November (Eq. 7), all the significant terms (A, AB, A^2, A^2B) had the same extent of impact, while in March (Eq. 8) the most important impacts were represented by the A term and the quadratic term A^2. In general, all experiments showed that the VS concentration had a strong impact, while the beating time had a relative minor impact on methane yield.
The final equations in terms of actual factors in May (Eq. 9), November (Eq. 10) and March (Eq. 11) respectively are reported below:

$$Y = + 35.88 + 107.33 A + 13.64 B + 2.17 AB - 29.53 A^2 - 0.68 B^2 \quad (9)$$

$$Y = + 648.05 - 268.79 A - 29.38 B + 17.39 AB + 39.74 A^2 - 2.52 A^2B \quad (10)$$

$$Y = + 14.05 + 117.35 A + 12.37 B - 33.43 A^2 - 0.45 B^2 \quad (11)$$

The resulting surfaces for each experiment and the correspondent contour plots are presented in Figures 2-3-4. All graphs showed better yields when the VS concentration was below 2.5%. Besides, both contour surfaces related to May and March presented a similar curvature with longer treatment times having a positive effect on the response. This kind of trend was not detected for the material harvested in November as the optimum region was characterised by a shorter treatment time.

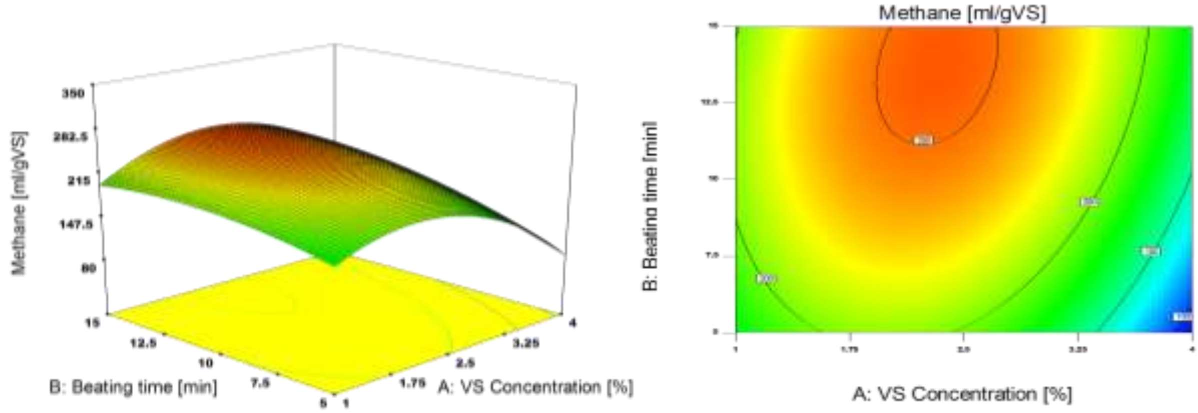


Figure 2: Response surface and contour plot of May 2014 experiment

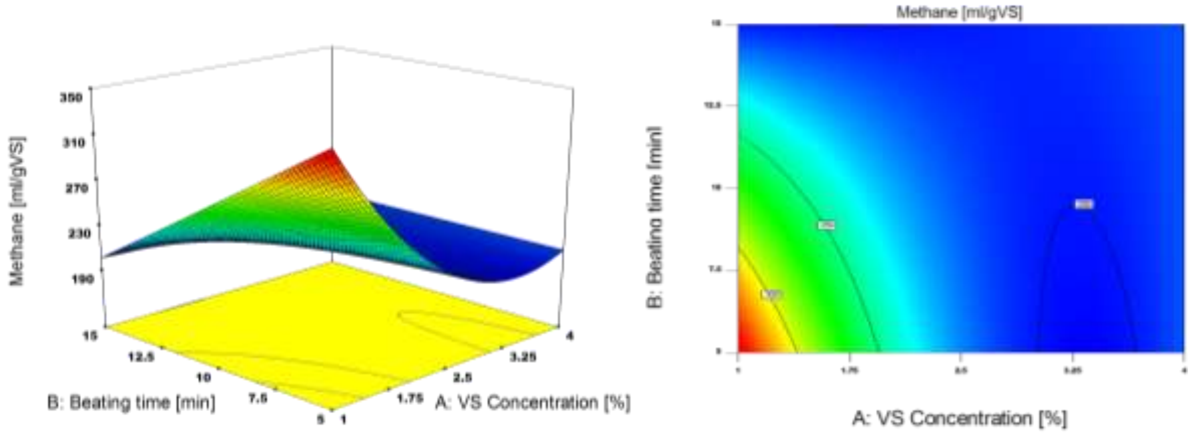


Figure 3: Response surface and contour plot of November 2014 experiment

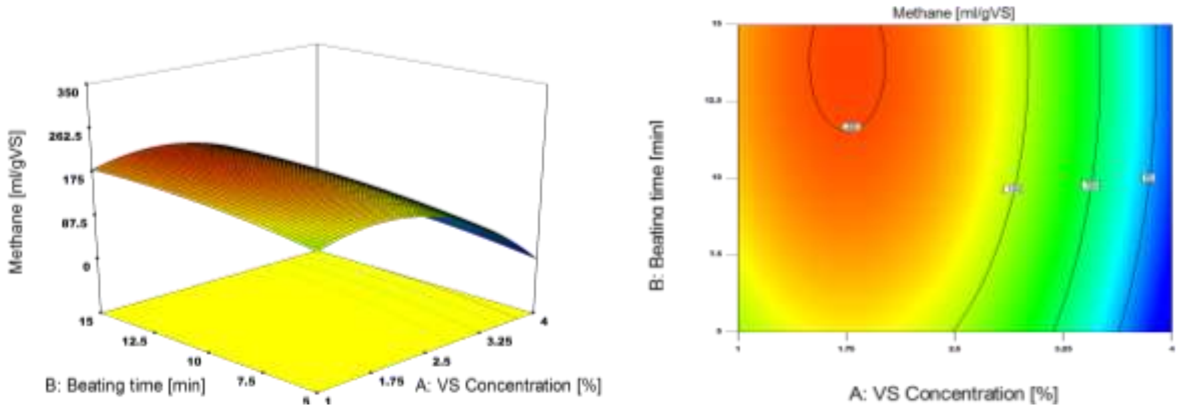


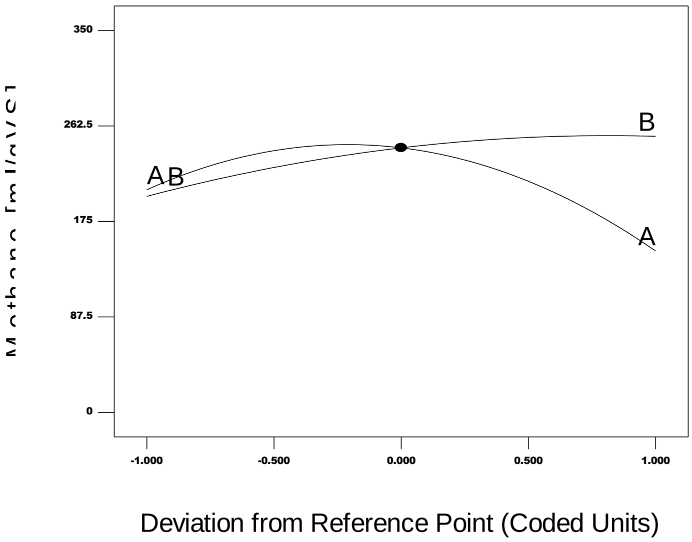
Figure 4: Response surface and contour plot of March 2015 experiment

The perturbation plots (Figure 5) showed similar trends for material harvested in May (Figure 5-a) and March (Figure 5-c), even though the methane yields were different. The curvature related to the VS concentration (A) meant that this factor impacted more on the response than the beating time (B). In particular, the methane yield was at the highest levels when the VS concentration was below the centre point (2.5%) while it decreased for higher values of VS.

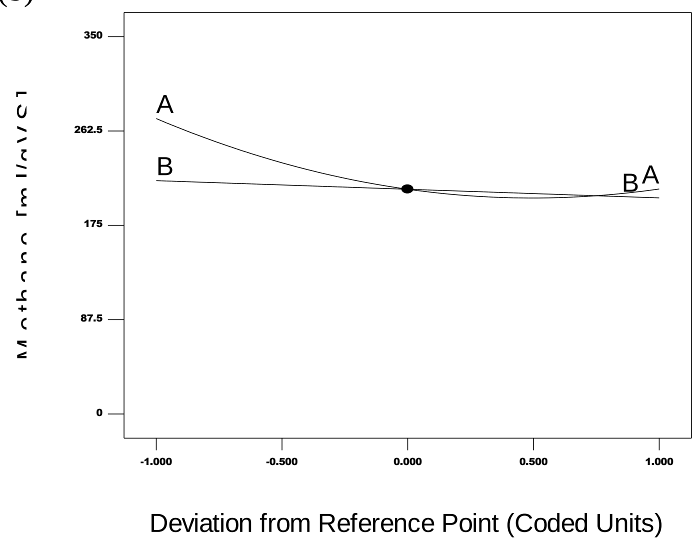
In November (Figure 5-b) there was a methane decrease while increasing the VS concentration over 2.5%; however such decrease resulted to be less important than in May and March.

Regarding the beating time, this had a stronger effect in May rather than in March, even though the general trend for these two months was an increase of methane with the time of pretreatment. Unlike May and March, the material harvested in November showed a decrease in methane yields while increasing the beating time. However the overall effect of the beating time was not statistically significant.

(a)



(b)



(c)

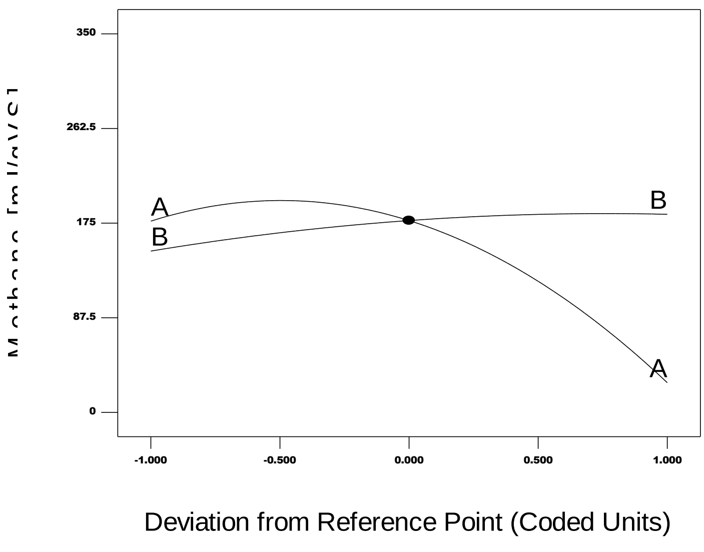
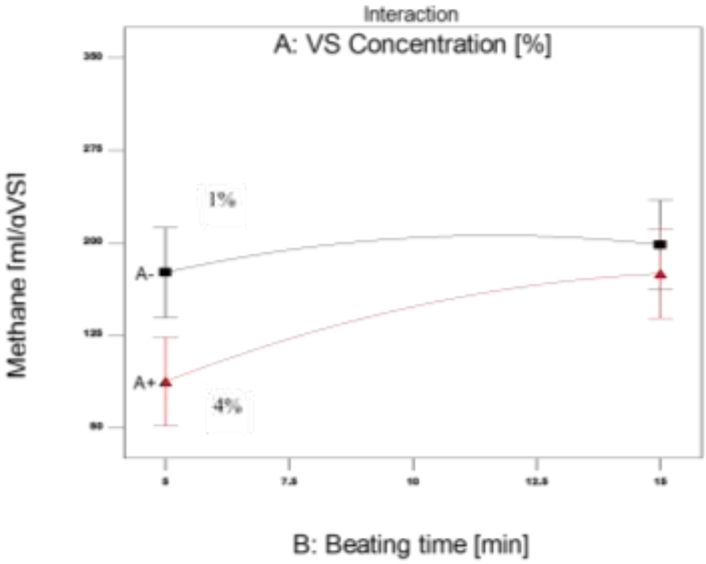


Figure 5: Perturbation plots in May 2014 (a), November 2014 (b) and March 2015 (c)

From the AB interaction plots (Figure 6) relative to May and November experiments, it was interesting to note that in both months, the response at 15 min was not affected by the VS concentration, while the predictions at 5 min of pretreatment were statistically significant. This means that when reducing the beating time up to 5 min, a reduction in VS concentration up to 1% was beneficial for the process, more in November than in May. When the VS concentration was set at 4%, in May an increase of beating time determined an increase of methane; while in November the pretreatment time did not have any significant effect.

(a)



(b)

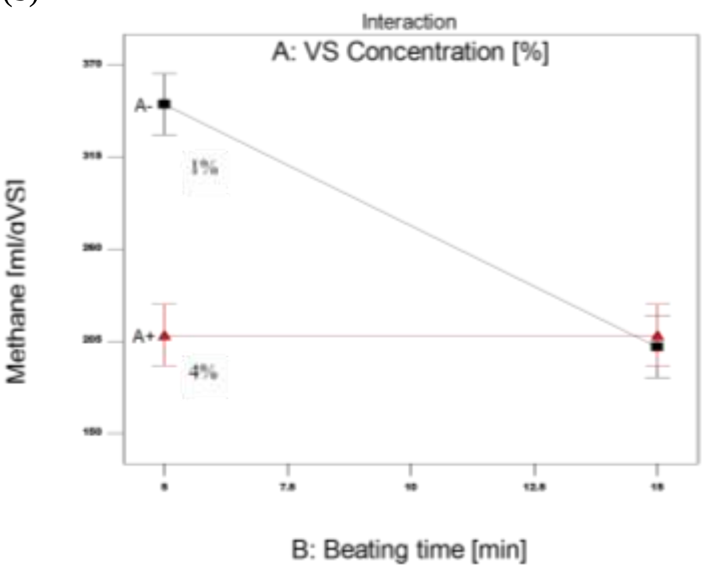


Figure 6: AB interaction plots in May (a), November (b)

3.3 Energy Evaluation

In general, AD from seaweeds was proven to be a valuable option from an energy point of view [42]. The use of a mechanical pretreatment is justified when it benefits the system by increasing the methane yield or lowering the digestion time. The achieved advantages must be large enough in order to make up for the energy consumed by the pretreatment and eventually generate more energy with respect to the scenario without pretreatment [43, 44].

Thus, a simple calculation based on the electricity consumption of the beating pretreatment measured during each experiment was carried out, according to *Section 2.6*.
The average methane perecentage measured for each experiment was used as biogas methane content. The machine energy consumption was measured during the experiment and expressed in Wh for gram of VS, according to the optimum VS concentration found for each experiment. The results are reported in Table 10.
According to this analysis, May was the only month during which the use of the beating pretreatment was not convenient, while a positive energy gain was achieved both in November and March. November and more so in general the autumn season, was the most suitable period to harvest *Laminaria sp.* for biogas production. In the same period, optimised conditions of VS concentration and beating time (1%, 5 min), would allow the highest increase in terms of energy production.
Even though the lowest methane yield was observed in March, the energy evaluation showed that there was a benefit in using the beating pretreatment. The extra methane produced after pretreatment with respect to the untreated sample, could make up for the energy consumed during the treatment and produce an extra energy of 26%.
If the biogas from seaweed biomass was used to produce only electricity at an efficiency of η=30% [45], only during autumn the use of beating pretreatment would cause an increase of energy up to 32% compared to the untreated scenario at optimised conditions.

Table 10: Energy evaluation experiments 2 (May 2014), 3 (November 2014) and 4 (March 2015)

	May	*November*	*March*
Treated			
Best treatment condition	VS=2.5% BT=15 min	VS=1% BT= 5 min	VS=2.5% BT= 15 min
Biogas produced [ml g^{-1}VS]	615	855	576
Average CH_4 [%]	39	40	37
B_S: Biogas energy content [kWh m^{-3}]	3.89	3.99	3.69
E_P: Produced energy [Wh g^{-1} VS]	2.39	3.41	2.12
E_P: Produced energy at η=30% [Wh g^{-1} VS]	0.72	1.02	0.64
E_C: Machine electricity consumption [Wh g^{-1} VS]	0.24	0.20	0.24
Net E_P: Net produced energy [Wh g^{-1} VS]	2.16	3.21	1.89
Net E_P: Net produced energy [Wh g^{-1} VS] at η=30%	0.48	0.82	0.40
Untreated			
Best untreated condition	VS=1%	VS=4%	VS=2.5%
Biogas produced [ml g^{-1}VS]	482	502	418
Average CH_4 [%]	50	41.6	35.9

B_S: Biogas energy content [kWh/m^3]	4.98	4.15	3.58
E_P: Produced energy [Wh g^{-1} VS]	2.40	2.08	1.50
E_P: Produced energy at η=30% [Wh g^{-1} VS]	0.72	0.62	0.45
Energy Gain/Loss [%]	-10	54	26
Energy Gain/Loss [%] at η=30%	-33	+32	-10

3.4 Discussion

Very few studies in the literature investigated the methane production through AD from *Laminaria sp.* at different periods of harvesting [35]. It is known that the seasonal fluctuation of *Laminaria sp.* components influences the methane conversion of this kind of seaweed [34, 35]. In Ireland, the influence of the organic substrate concentration and the pretreatment phase when harvesting the seaweed at different periods of the year was not investigated to date. In general, the main carbohydrates in *Laminaria sp.* are mannitol, laminarin and alginic acid. Alginic acid, also called alginate, is a polysaccharide widely distributed in the cell walls of brown seaweed while laminarin and mannitol are the major carbon storage compounds in monomeric (mannitol) or polymeric (laminarin) form [46]. During AD, mannitol is utilised more efficiently than the polymers laminarin and alginic acid [35]. According to Schiener *et al.* [34], the average mannitol content in *Laminaria digitata*, *Laminaria hyperborea* and *Saccharina latissima* was at 19.4 ± 6.6, 17.5 ± 7.4, and 18.6 ± 4.7%, respectively and the average laminarin content for the same species accounted for 6.7 ± 6.0, 7.4 ± 8.0, 8.2 ± 5.3 % of the dry weight. During autumn, the highest mannitol levels of 24-27% were observed while the lowest levels of 6–8 % were recorded in early spring. Laminarin followed a similar trend rising to its highest levels during the summer and autumn months (25 % max. in *Laminaria hyperborea*) and dropping to its lowest levels (1-3 %) in winter [34]. Alginate formed the majority of the carbohydrate content accounting for 34.6 ± 3.1, 33.2 ± 3.8 and 28.5 ± 3.9 % of the dry weight in *Laminaria digitata*, *Laminaria hyperborea* and *Saccharina latissima*, respectively. In accordance with the levels of mannitol and laminarin reported by Schiener *et al.* [34], the highest yields of methane were recorded during autumn (November) which corresponded to the peak for laminarin and mannitol content, while the lowest recordings corresponded to early spring (March), when the carbohydrates content was reported at its minimum. In a study conducted in the UK by Adams *et al.* [35], the highest methane yield of 254.14 ± 6.21ml g^{-1} VS was reported in July when the macroalgae presented the highest combined proportion of mannitol and laminarin in conjunction with lowest concentration of ash and alkali metals. The current study found out a higher methane yield up to 35% of extra methane in November, by using the beating pretreatment for 5 min and at 1% of VS. According to Adams *et al.*'s result [35], July represented the best month for harvesting while in this investigation higher methane yields were achieved in November, by optimising the beating pretreatment and the VS concentration. However, it must be noticed that this study did not consider an experiment in July. It could be interesting to apply the same experimental conditions for material harvested in July in order to verify if it is possible to reach a further increase in methane yield. During November the statistical analysis estimated that the joint action of the VS concentration and the beating time affected significantly the methane response. The RSM analysis showed that a reduction of beating time up to 5 min determines a dramatic enhancement of methane at 1% of VS, which is not detected at 4% of

VS. This meant that during autumn, it was necessary to vary these two factors simultaneously in order to optimise the process.
This study reported the lowest yields in March in accordance with Adams *et al.* [35]. The best yield of 215 ± 9 ml CH_4 g^{-1} VS was measured at 2.5% of VS and after 15 min of beating treatment with an increase of almost 10% with respect to Adams *et al.*'s result [35]. During this month, *Laminaria sp.* is generally characterised by low concentrations of carbohydrates. In proportion, high concentrations of alginic acid were observed in conjunction with low mannitol and laminarin concentrations. Alginic acid is known to have a slower hydrolysation rate than mannitol and laminarin [35]. Therefore, low levels of mannitol, laminarin, and slow alginate hydrolysation rate were likely to be the reasons of the lower methane yields observed during this month. Unlike other months, in March, the only parameter which had a strong impact on the methane response was the VS concentration. This was the only experiment characterised by a severe failure of the digester at 4% of VS. This suggested that in March, the choice of the VS concentration was a major issue in order to optimise the process.
In May, an increased methane yield of 14% with respect to Adams *et al.*'s result [35] was observed after 10 min of pretreatment and at 2.5% of VS. However, it must be noticed that it was not observed much improvement with respect to the untreated sample. The yields during this period resulted to be higher than those registered in March, but still lower than the yields measured in autumn. This trend is also confirmed by Adams *et al.*'s work [35]. Also in this case, the methane yield reflected the levels of the algal carbohydrates, which were observed to be not as high as during autumn and not so low as in winter or early spring.
In general, for all the harvesting periods, the higher methane yields were observed at an optimum VS concentration below 2.5%. Autumn was the best harvesting period in order to exploit *Laminaria sp.* as feedstock for AD. During this period, the system would benefit the most by applying the beating pretreatment. The energy balance calculated an energy gain of 54%, in accordance with Tedesco *et al.* [47] who also used the beating pretreatment. In particular, short pretreatment times were sufficient in order to obtain the best methane yields; this would also be beneficial for the economics of the process. Even though early spring represented the worst period for harvesting *Laminaria sp.*, it was possible to improve the system performance by applying the beating pretreatment and optimising the VS concentration. In particular, an interesting finding is that the extra methane produced after pretreatment could make up for the energy consumed during the treatment and produce an extra energy of 26%.
In this discussion, it was underlined that the seaweed carbohydrates levels affect the methane production. For a better understanding of such matter, the next section provides a brief discussion about the Irish environmental factors which seasonally affect the seaweed carbohydrates levels.

3.4.1 Discussion on environmental factors

It is known that amongst the major environmental factors affecting seaweeds chemical composition are light, temperature, salinity, water motion and nutrient availability [48]. High light intensities increase the rate of photosynthesis and the polysaccharide production [49] and a positive correlation exists between temperature and carbohydrates content [50]. Light quantity and quality depend on season, depth and turbidity. The turbidity affects negatively the seaweeds carbohydrates content since determines a reduction in irradiance [48, 51]. This factor is influenced by fast tidal motions [52], nutrient availability and pollution [48]. In particular, the Irish Sea is characterised by very high turbid seawater, especially during winter [52, 53] due to the strongest winds generally registered in this season. The data regarding the solar radiation registered in Dublin over several years revealed a peak between May and August, while declining from December up to February (Table 11). Regarding the sea temperature, highest temperatures were registered between July and November and the lowest between winter and spring (Figure 7). In this case, it is worth noting that highest and lowest temperatures occur later in the year at sea than overland since water takes longer to warm up and cool down. In general, sea temperatures are higher than those of the air during winter, while the reverse is the case during summer months. By comparing the air temperatures with sea temperatures [54, 55], the temperature trends are shift of one month between each other. For instance, while for the air temperature the peak is generally registered in July, for sea temperatures the peak is registered in August.

Table 11: Global Solar Radiation in Joules cm^{-2} for Dublin [55]

	Year				
Month	*2012*	*2013*	*2014*	*2015*	*Mean*
Jan	7580	5909	6508	8749	7228
Feb	12456	12106	14654	13203	12761
Mar	28991	19993	25421	29537	25705
Apr	37313	40281	42869	47485	39407
May	51564	55706	45343	51364	52530
Jun	46884	59657	57067	60157	52648
Jul	48889	61855	54042	48312	50860
Aug	40767	43342	42419	44488	42506
Sep	33093	31714	31993	32290	30043
Oct	16838	15960	19354	17342	18168
Nov	10753	10184	8050	7486	8935
Dec	6187	6146	6317	4743	5550
Annual	341315	362853	354037	365156	346340

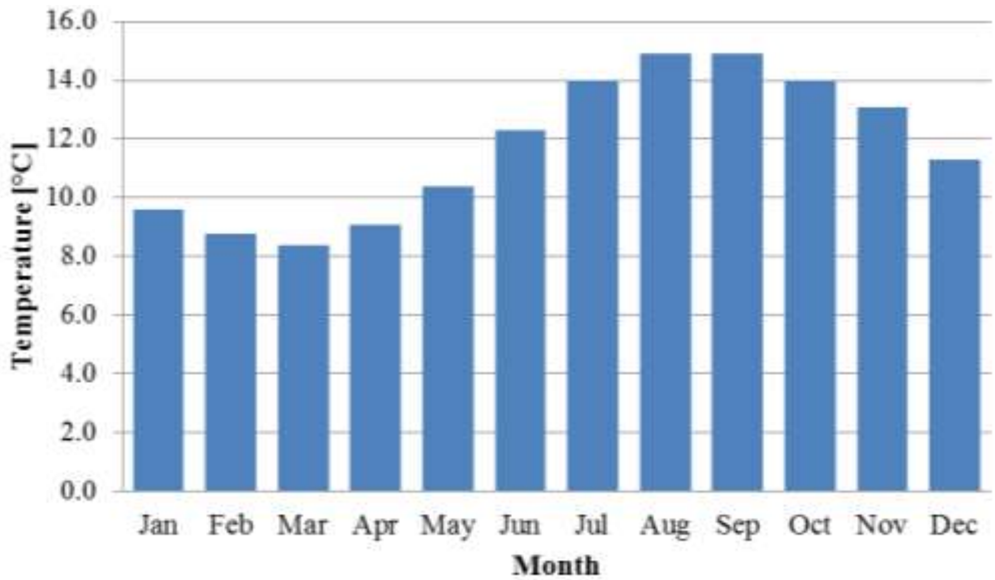


Figure 7: Average sea temperatures for Dublin over several years of archived data [54]

According to these data, it is likely that the seaweed biomass stores the main carbohydrates during summer due to high solar radiations and temperatures, and then consumes them during winter for tissue growth. Therefore, by considering the Irish climate, summer and autumn are in general the best harvesting periods of *Laminaria sp.* for bioenergy exploitation, since the seaweed is rich of carbohydrates stored during summer months thanks to higher solar radiation and temperatures that are generally recorded in these months, while in early spring (March) the biomass is poor of nutrients due to the consumption during winter months.

May is generally characterised by high solar radiation and high sea temperatures. In this case though, methane yields as high as in autumn were not observed probably due to the fact that the seaweed had not already reached high levels of carbohydrates. This resulted in higher TS (19 ± 2% Wt on wet basis), VS (84 ± 1% of TS) contents and methane production during November, while lower levels were seen in March and May, with May having a higher VS content standard deviation, as during this period the seaweed was starting to accumulate nutrients.

Therefore, according to the low levels of algal carbohydrates both in winter and early spring, it is likely that the winter months would be characterised by similar yields as those observed in March. On the other hand, both summer and autumn are characterised by high levels of carbohydrates, thus it is possible that during summer similar yields as in November are likely to be observed.

4. CONCLUSIONS

This research aimed to select the best harvesting period of beach cast brown seaweed *Laminaria sp*. for methane production, while optimising the use of a beating pretreatment and the organic substrate concentration. For all the harvesting periods, the higher methane yields were observed at an optimum VS concentration below 2.5%. Autumn appeared the best harvesting period. During this period, the system would benefit the most by applying the beating pretreatment. In particular, short pretreatment times were sufficient in order to obtain the best methane yields of 342±17 ml g^{-1} VS, at the lowest organic substrate concentration (1% VS). Even though early spring represented the worst period for harvesting *Laminaria sp.*, the use of the beating pretreatment at a VS concentration of 2.5% allowed a methane production 47% higher than the untreated sample. In November, the energy balance showed the highest energy gain of 54% after pretreatment, while an energy loss of 10% was registered in May.

5. ACKNOWLEDGEMENT

This research was supported by the Irish Research Council for Science, Engineering and Technology (IRCSET), funded by the National Development Plan (RS/2012/417).

References

[1] Plevin, R.J., Jones, A.D., Torn, M.S. and Gibbs, H.K., 2010. Greenhouse gas emissions from biofuels’ indirect land use change are uncertain but may be much greater than previously estimated. *Environmental Science & Technology*, *44*(21), pp.8015-8021.

[2] Roberts, T. and Upham, P., 2012. Prospects for the use of macro-algae for fuel in Ireland and the UK: an overview of marine management issues. *Marine Policy*, *36*(5), pp.1047-1053.

[3] Nigam, P.S. and Singh, A., 2011. Production of liquid biofuels from renewable resources. *Progress in energy and combustion science*, *37*(1), pp.52-68.

[4] Chisti, Y., 2007. Biodiesel from microalgae. *Biotechnology advances*, *25*(3), pp.294-306.

[5] Daroch, M., Geng, S. and Wang, G., 2013. Recent advances in liquid biofuel production from algal feedstocks. *Applied Energy*, *102*, pp.1371-1381.

[6] Demirbas, M.F., Balat, M. and Balat, H., 2009. Potential contribution of biomass to the sustainable energy development. *Energy Conversion and Management*, *50*(7), pp.1746-1760.

[7] Hughes, A.D., Kelly, M.S., Black, K.D. and Stanley, M.S., 2012. Biogas from Macroalgae: is it time to revisit the idea?. *Biotechnology for biofuels*, *5*(1), p.1.

[8] Wargacki, A.J., Leonard, E., Win, M.N., Regitsky, D.D., Santos, C.N.S., Kim, P.B., Cooper, S.R., Raisner, R.M., Herman, A., Sivitz, A.B. and Lakshmanaswamy, A., 2012. An engineered microbial platform for direct biofuel production from brown macroalgae. *Science*, *335*(6066), pp.308-313.

[9 Burton, T., Lyons, H., Lerat, Y., Stanley, M. and Rasmussen, M.B., 2009. *A review of the potential of marine algae as a source of biofuel in Ireland*. Dublin: Sustainable Energy Ireland-SEI.

[10] Browne, J., Nizami, A.S., Thamsiriroj, T. and Murphy, J.D., 2011. Assessing the cost of biofuel production with increasing penetration of the transport fuel market: A case study of gaseous biomethane in Ireland. *Renewable and Sustainable Energy Reviews*, *15*(9), pp.4537-4547.

[11] Langlois, J., Sassi, J.F., Jard, G., Steyer, J.P., Delgenes, J.P. and Hélias, A., 2012. Life cycle assessment of biomethane from offshore-cultivated seaweed. *Biofuels, Bioproducts and Biorefining*, *6*(4), pp.387-404.

[12] Singh, A., Smyth, B.M. and Murphy, J.D., 2010. A biofuel strategy for Ireland with an emphasis on production of biomethane and minimization of land-take. *Renewable and Sustainable Energy Reviews*, *14*(1), pp.277-288.

[13] Smyth, B.M., Smyth, H. and Murphy, J.D., 2010. Can grass biomethane be an economically viable biofuel for the farmer and the consumer?. *Biofuels, Bioproducts and Biorefining*, *4*(5), pp.519-537.

[14] Smyth, B.M., Murphy, J.D. and O’Brien, C.M., 2009. What is the energy balance of grass biomethane in Ireland and other temperate northern European climates?. *Renewable and Sustainable Energy Reviews*, *13*(9), pp.2349-2360.

[15] McEniry, J., Crosson, P., Finneran, E., McGee, M., Keady, T.W.J. and O'Kiely, P., 2013. How much grassland biomass is available in Ireland in excess of livestock requirements?. *Irish Journal of Agricultural and Food Research*, pp.67-80.

[16] O'Donovan, M., Lewis, E. and O'Kiely, P., 2011. Requirements of future grass-based ruminant production systems in Ireland. *Irish Journal of Agricultural and Food Research*, pp.1-21.

[17] Allen, E., Wall, D.M., Herrmann, C., Xia, A. and Murphy, J.D., 2015. What is the gross energy yield of third generation gaseous biofuel sourced from seaweed?. *Energy*, *81*, pp.352-360.

[18] Hughes, A.D., Black, K.D., Campbell, I., Heymans, J.J., Orr, K.K., Stanley, M.S. and Kelly, M.S., 2013. Comments on 'prospects for the use of macroalgae for fuel in Ireland and UK: an overview of marine management issues'. *Marine Policy*, *38*, pp.554-556.

[19] Department of Agriculture, Food and the Marine. 2020 marine sector bioeconomy. *Irish Marine Institute*. 2013.

[20] Murphy, F., Devlin, G., Deverell, R. and McDonnell, K., 2013. Biofuel production in Ireland—an approach to 2020 targets with a focus on algal biomass. *Energies*, *6*(12), pp.6391-6412.

[21] Montingelli, M.E., Tedesco, S. and Olabi, A.G., 2015. Biogas production from algal biomass: a review. *Renewable and Sustainable Energy Reviews*, *43*, pp.961-972.

[22] Singh, A. and Olsen, S.I., 2011. A critical review of biochemical conversion, sustainability and life cycle assessment of algal biofuels. *Applied Energy*,*88*(10), pp.3548-3555.

[23] Ghadiryanfar, M., Rosentrater, K.A., Keyhani, A. and Omid, M., 2016. A review of macroalgae production, with potential applications in biofuels and bioenergy. *Renewable and Sustainable Energy Reviews*, *54*, pp.473-481.

[24] Chisti, Y. and Yan, J., 2011. Energy from algae: current status and future trends: algal biofuels–a status report. *Applied Energy*, *88*(10), pp.3277-3279.

[25] Dębowski, M., Zieliński, M., Grala, A. and Dudek, M., 2013. Algae biomass as an alternative substrate in biogas production technologies—review. *Renewable and Sustainable Energy Reviews*, *27*, pp.596-604.

[26] Vanegas, C.H. and Bartlett, J., 2013. Green energy from marine algae: biogas production and composition from the anaerobic digestion of Irish seaweed species. *Environmental technology*, *34*(15), pp.2277-2283.

[27] Taherzadeh, M.J. and Karimi, K., 2008. Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. *International journal of molecular sciences*, *9*(9), pp.1621-1651.

[28] Nkemka, V.N. and Murto, M., 2012. Exploring strategies for seaweed hydrolysis: Effect on methane potential and heavy metal mobilisation. *Process Biochemistry*, *47*(12), pp.2523-2526.

[29] Tedesco, S., Barroso, T.M. and Olabi, A.G., 2014. Optimization of mechanical pre-treatment of Laminariaceae spp. biomass-derived biogas.*Renewable Energy*, *62*, pp.527-534.

[30] Montingelli, M.E., Benyounis, K.Y., Stokes, J. and Olabi, A.G., 2016. Pretreatment of macroalgal biomass for biogas production. *Energy Conversion and Management*, *108*, pp.202-209.

[31] Hanssen, J.F., Indergaard, M., Østgaard, K., Bævre, O.A., Pedersen, T.A. and Jensen, A., 1987. Anaerobic digestion of Laminaria spp. and Ascophyllum nodosum and application of end products. *Biomass*, *14*(1), pp.1-13.

[32] Ehimen, E.A., Sun, Z.F., Carrington, C.G., Birch, E.J. and Eaton-Rye, J.J., 2011. Anaerobic digestion of microalgae residues resulting from the biodiesel production process. *Applied Energy*, *88*(10), pp.3454-3463.

[33] George, T., Franklin, L.B. and Stensel, H.D., 2003. Wastewater engineering; Treatment and Reuse. *New York: Metcalf & Eddy*.

[34] Schiener, P., Black, K.D., Stanley, M.S. and Green, D.H., 2015. The seasonal variation in the chemical composition of the kelp species Laminaria digitata, Laminaria hyperborea, Saccharina latissima and Alaria esculenta. *Journal of Applied Phycology*, *27*(1), pp.363-373.

[35] Adams, J.M.M., Toop, T.A., Donnison, I.S. and Gallagher, J.A., 2011. Seasonal variation in Laminaria digitata and its impact on biochemical conversion routes to biofuels. *Bioresource technology*, *102*(21), pp.9976-9984.

[36] VDI V. 4630 - Fermentation of organic materials. Characterisation of Substrate, Sampling, Collection of Material Data, Fermentation Tests [1872] VDI *The Association of German Engineers*. 2006.

[37] Sluiter, A., Hames, B., Hyman, D., Payne, C., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D. and Wolfe, J., 2008. Determination of total solids in biomass and total dissolved solids in liquid process samples. *National Renewable Energy Laboratory, Golden, CO, NREL Technical Report No. NREL/TP-510-42621*, pp.1-6.

[38] Ehrman, T., 1994. Standard Method for Ash in Biomass, Laboratory Analytical Procedure-005. *National Renewable Energy Laboratory, Golden, CO*.

[39] Eriksson, O., 2010. Environmental technology assessment of natural gas compared to biogas. *INTECH Open Access Publisher*.

[40] Swedish Gas Center, 2012. Basic data on biogas. *Available Online, Accessed January 2016*.

[41] Hobson, P.N. and Wheatley, A.D., 1993. *Anaerobic digestion: modern theory and practice*. London: Elsevier applied science.

[42] Anastasakis, K. and Ross, A.B., 2015. Hydrothermal liquefaction of four brown macro-algae commonly found on the UK coasts: an energetic analysis of the process and comparison with bio-chemical conversion methods. *Fuel*,*139*, pp.546-553.

[43] Cheng, J.J. and Timilsina, G.R., 2011. Status and barriers of advanced biofuel technologies: A review. *Renewable Energy*, *36*(12), pp.3541-3549.

[44] Kratky, L. and Jirout, T., 2011. Biomass size reduction machines for enhancing biogas production. *Chemical Engineering & Technology*, *34*(3), pp.391-399.

[45] Pöschl, M., Ward, S. and Owende, P., 2010. Evaluation of energy efficiency of various biogas production and utilization pathways. *Applied Energy*, *87*(11), pp.3305-3321.

[46] Anastasakis, K., Ross, A.B. and Jones, J.M., 2011. Pyrolysis behaviour of the main carbohydrates of brown macro-algae. *Fuel*, *90*(2), pp.598-607.

[47] Tedesco, S., Benyounis, K.Y. and Olabi, A.G., 2013. Mechanical pretreatment effects on macroalgae-derived biogas production in co-digestion with sludge in Ireland. *Energy*, *61*, pp.27-33.

[48] Hurd, C.L., Harrison, P.J., Bischof, K. and Lobban, C.S., 2014. *Seaweed ecology and physiology*. Cambridge University Press.

[49] Hu, Q., 2004. 5 Environmental Effects on Cell Composition. *Handbook of microalgal culture: biotechnology and applied phycology*, p.83.

[50] Marinho-Soriano, E., Fonseca, P.C., Carneiro, M.A.A. and Moreira, W.S.C., 2006. Seasonal variation in the chemical composition of two tropical seaweeds. *Bioresource Technology*, *97*(18), pp.2402-2406.

[51] Werner, A., Clarke, D. and Kraan, S., 2004. Strategic Review and the Feasibility of Seaweed Aquaculture in Ireland.

[52] White, M., Gaffney, S., Bowers, D.G. and Bowyer, P., 2003, August. Interannual variability in Irish Sea turbidity and relation to wind strength. In *Biology and Environment: Proceedings of the Royal Irish Academy* (pp. 83-90). Royal Irish Academy.

[53] Bowers, D.G., Gaffney, S., White, M. and Bowyer, P., 2002. Turbidity in the southern Irish Sea. *Continental Shelf Research*, *22*(15), pp.2115-2126.

[54] World Sea Temperatures. Dublin Sea Temperature. Available online at www.seatemperature.org. Updated 2016. Accessed January, 2016.

[55] MET ÉIREANN. Weather data. Available online at www.met.ie. Updated 2016. Accessed January, 2016.